Progress in Nuclear Magnetic Resonance Spectroscopy 77 (2014) 49–68

Contents lists available at ScienceDirect

Progress in Nuclear Magnetic Resonance Spectroscopy

journal homepage: www.elsevier.com/locate/pnmrs

# Indirect use of deuterium in solution NMR studies of protein structure and hydrogen bonding

Vitali Tugarinov *

*Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, United States*

Edited by J. Feeney and J.W. Emsley

ARTICLE INFO

*Article history:*
Received 1 July 2013
Accepted 15 August 2013
Available online 28 August 2013

*Keywords:*
Deuteration
Deuterium isotope shifts
Spin relaxation in deuterium-containing spin-systems
Protein backbone geometry
Protein dynamics
Hydrogen bonds

ABSTRACT

A description of the utility of deuteration in protein NMR is provided with an emphasis on quantitative evaluation of the effects of deuteration on a number of NMR parameters of proteins: (1) chemical shifts, (2) scalar coupling constants, (3) relaxation properties ($R_1$ and $R_2$ rates) of nuclei directly attached to one or more deuterons as well as protons of methyl groups in a highly deuterated environment, (4) scalar relaxation of $^{15}N$ and $^{13}C$ nuclei in $^{15}N$–D and $^{13}C$–D spin systems as a measure of hydrogen bonding strength, and (5) NOE-based applications of deuteration in NMR studies of protein structure. The discussion is restricted to the 'indirect' use of deuterium in the sense that the description of NMR parameters and properties of the nuclei affected by nearby deuterons ($^{15}N$, $^{13}C$, $^{1}H$) is provided rather than those of deuterium itself.



**Contents**



## 1. Introduction

Over the past two decades, deuteration has evolved into an important tool for solution NMR studies of proteins and protein complexes [1–7]. Besides direct effects of deuteration on $^{13}C$ line

*Abbreviations:* TROSY, transverse relaxation optimized spectroscopy; BIRD, bilinear rotation decoupling; CPMG, Carr–Purcell–Meiboom–Gill pulse train; QCC, quadrupolar coupling constant; CSA, chemical shift anisotropy; RDC, residual dipolar coupling; NOE, nuclear Overhauser effect; MSG, Malate Synthase G.

* Address: Biomolecular Sci. Bldg., Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, United States. Tel.: +1 301 4051504; fax: +1 301 3140386.
*E-mail address:* vitali@umd.edu

0079-6565/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.pnmrs.2013.08.001

widths [8,9], all types of transverse relaxation optimized spectroscopy (TROSY) [10,11] benefit from perdeuteration of protein molecules. Deuteration is of paramount importance for methyl-TROSY spectroscopy of selectively protonated $^{13}CH_3$ groups [12,13], as the line-widths of the slow-relaxing components of methyl $^1H$–$^{13}C$ multiple-quantum coherences are very sensitive to the deuteration levels of the rest of the protein structure [12,14,15].

The effects of deuteration on several NMR parameters in proteins have been reported: one-bond $^1H$–$^{13}C$ scalar couplings in methyl groups [16], equilibrium proton/deuterium fractionation factors for backbone amides [17], deuterium isotope shifts [18–24], as well as the relaxation properties of backbone amide $^{15}N$ nuclei in $^{15}N$–D [25,26] and $^{13}C$ nuclei in $^{13}C_\alpha$–$D_\alpha$ [27] spin pairs. Even though $^{13}C$ relaxation studies of bio-macromolecules have a three decade long history [28–35], quantitative evaluation of the effects of deuteration on relaxation properties of $^{13}C$ nuclei are less common. Grzesiek and Bax [36] and London et al. [37] have described multiplet structures of $^{13}C$ nuclei attached to deuterium. The utilities of relaxation properties of $^{13}C$ in $^{13}C^\alpha H^\alpha D^\alpha$ spin-systems of glycines [38] and $^{13}C_\alpha$–$D_\alpha$ spin pairs of proteins [27] have been assessed.

Here, we provide quantitative descriptions of the effects of deuteration on a number of NMR parameters in proteins: (i) chemical shifts, (ii) coupling constants, (iii) relaxation properties ($R_1$ and $R_{1\rho}/R_2$ rates) of nuclei directly attached to one or more deuterons as well as protons of methyl groups in a highly deuterated environment, (iv) scalar relaxation of $^{15}N$ and $^{13}C$ nuclei in $^{15}N$–D and $^{13}C$–D spin systems as a measure of hydrogen bonding strength, and (v) nuclear Overhauser effect (NOE)-based applications of deuteration in NMR studies of protein structure and hydrogen bonding. These effects are considered 'indirect' because the NMR parameters and properties of the nuclei affected by nearby deuterons are quantified ($^{15}N$, $^{13}C$, $^1H$) rather those of deuterium itself. Direct measurements of spin relaxation and quadrupolar coupling constants of deuterium in various spin systems encountered in proteins ($^{15}NHD$, $^{13}C_\alpha D$, $^{13}C_\beta HD$ and methyl $^{13}CH_2D/^{13}CHD_2$ groups) pioneered by Kay and co-workers [39–46] are outside the scope of this review.

## 2. The changes in NMR parameters of proteins resulting from deuteration

### 2.1. Deuterium isotope shifts of the backbone and $^{13}C_\beta$ nuclei of proteins

The importance of the changes in isotropic chemical shielding of nuclei removed one or more bonds away from the site of a proton-to-deuterium ($^1H$-to-D) substitution (deuterium isotope shifts [47,48]) in peptides and proteins has been recognized for more than two decades [49–55]. The effects of $^1H$-to-D replacement at exchangeable sites of peptides and proteins tend to be isolated (i.e. separated from each other by multiple bonds in the polypeptide chain), and have been the focus of most of initial investigations of deuterium isotope shifts. In particular, the effects of $^1H$-to-D substitutions at exchangeable sites of backbone amides ($^{15}N$–$^1H$/D), tyrosine hydroxyl (O–$^1H$/D) or cysteine sulfhydryl groups (S–$^1H$/D) on the chemical shifts of carbonyl carbons [49–54], $^{13}C_\alpha$ and $^{13}C_\beta$ nuclei [20,56,57], as well as tyrosine $^{13}C_\zeta$ [58] and cysteine $^{13}C_\beta$ sites [22] have been reported. Hydrogen-bonding effects on one-bond isotope shifts of backbone $^{15}N$ [21,59], side-chain $^{15}N$ of asparagine and glutamine residues [60], $^{15}N/^1H_N$ nuclei in $NH_3$ groups of lysines [61] and even $^1H$ four-bond isotope shifts in phospholipase A2 [62] have been investigated.

Deuterium isotope effects arising from $^1H$-to-D substitutions at aliphatic carbon sites are more difficult to quantify because these effects are usually cumulative as all the aliphatic carbon positions are usually deuterated simultaneously leading to the additive effects of multiple $^1H$-to-D replacements [55,63]. Although these deuterium isotope shifts may complicate assignments of protein resonances [19,64–66] and, in the case of partial deuteration, also increase $^{13}C$ line-widths, the knowledge of their accurate values is equally important for better understanding of their physical and structural origins. These isotope effects have been measured in several studies by comparison of chemical shifts in fully protonated and fully deuterated protein molecules [8,9,19,64]. LeMaster and co-workers used differential one-bond deuterium isotope shifts of glycine $^{13}C_\alpha$ nuclei (the differences in $^{13}C_\alpha$ chemical shifts between glycine methylenes deuterated at pro-*R* and pro-*S* sites to establish conformational preferences of glycine residues in proteins [18]. Recently, Bax and co-workers have devised a computational approach to 'de-convolute' multiple contributions to deuterium isotope shifts of all backbone nuclei arising from the deuteration of non-exchangeable aliphatic sites of an intrinsically disordered protein α-synuclein [23].

An experimental approach towards determining precise deuterium isotope shifts of protein backbone nuclei ($^{13}C_\alpha$, $^{15}N$, $^{13}CO$, and $^1HN$) arising from $^1H$-to-D substitutions at non-exchangeable sites has been described by Sun and Tugarinov [24]. This approach is based on the application of multiple (two or more) biliniear rotation decoupling (BIRD) filtering elements [67,68] for isolation of molecular species with defined protonation/deuteration patterns at successive $^{13}C_\alpha$ nuclei in the polypeptide chain of a protein and allows one to distinguish and accurately quantify a variety of isotope effects within the protein backbone [24]. This methodology has been later extended to the measurements of deuterium isotope effects resulting from $^1H_N \rightarrow D_N$ substitutions at backbone amide positions on the chemical shifts of $^{15}N$, $^{13}C_\alpha$, $^{13}CO$, $^{13}C_\beta$ and $^1H_\alpha$ nuclei in proteins [69]. Fig. 1 shows the fragments of a protein polypeptide chain in various states of protonation/deuteration at aliphatic carbon positions ($^{13}C_\alpha$ and $^{13}C_\beta$) (Fig. 1A) and backbone amide sites (Fig. 1B), while Fig. 2 provides diagrams of the 2D *intra*-HN[CA/CO/N] experimental scheme used for accurate measurements of deuterium isotope shifts resulting from $^1H$-to-D substitution at aliphatic carbon sites (Fig. 2A), and the 2D *intra*-HA[CA/CO/N/CB] scheme [69] designed for precision measurements of deuterium isotope effects arising from $^1H$-to-D replacement at backbone amide positions (Fig. 2B). At the crux of this experimental approach is the creation of magnetization terms that include both the $^{13}C_\alpha(^{15}N)$ operators of the current residue ($i$) and the preceding(following) residue $i-1(i+1)$: i.e. the terms $N_{z,i}CO_{z,i-1}C^\alpha_{y,i-1}C^\alpha_y$ and $C^\alpha_{z,i}CO_{z,i}N_{y,i+1}N_{y,i}$ in the 2D *intra*-HN[CA/CO/N] and *intra*-HA[CA/CO/N/CB] experiments, respectively [24,69–71]. Then, depending on which type of the four isotopic species these terms belong to ($D_{\alpha,i-1}D_{\alpha,i}$ or $H_{\alpha,i-1}D_{\alpha,i}$ or $D_{\alpha,i-1}H_{\alpha,i}$ or $H_{\alpha,i-1}$-$H_{\alpha,i}$ in the *intra*-HN[CA/CO/N] scheme, and $D_{N,i}D_{N,i+1}$ or $H_{N,i}D_{N,i+1}$ or $D_{N,i}H_{N,i+1}$ or $H_{N,i}H_{N,i+1}$ in the *intra*-HA[CA/CO/N/CB] scheme; leftmost column in Fig. 2A and B), the terms will either retain their sign or change their sign after the application of the first BIRD [67,68] filtering element ($\boldsymbol{F}_1$) as it is shown in Fig. 2A and B. This allows their differentiation via the phase-cycling of the phase $\phi_1$ together with the receiver phase. Subsequently, the terms $N_{z,i}CO_{z,i-1}C^\alpha_{y,i-1}C^\alpha_y$ and $C^\alpha_{z,i}CO_{z,i}N_{y,i+1}N_{y,i}$ are converted respectively to those containing only the operators $^{13}C_\alpha(^{15}N)$ of the current residue ($i$): $N_{z,i}CO_{z,i-1}C^\alpha_y$ and $C^\alpha_{z,i}CO_{z,i}N_{y,i}$. Again, depending on the isotopic content of the molecular species at residue $i$, these latter operators will change or retain their sign after the application of the second BIRD filter ($\boldsymbol{F}_2$) and concomitant cycling of the phase $\phi_2$ and the receiver allowing for the selection of only a single isotopic species (rightmost column in Fig. 2A and B) from the two pairs of possibilities. Thus, double-filtering achieved by the elements $\boldsymbol{F_1}$ and $\boldsymbol{F_2}$ ensures isolation of NMR signals corresponding to each of the four types of isotopic species into four separate